Marinobacter algicola sp. nov., isolated from laboratory cultures of paralytic shellfish toxin-producing dinoflagellates

David H. Green,¹ John P. Bowman,² Elizabeth A. Smith,³ Tony Gutierrez¹ and Christopher J. S. Bolch⁴

Correspondence

David H. Green

david.green@sams.ac.uk

¹Scottish Association for Marine Science, Dunstaffnage Marine Laboratory, Oban, Argyll PA37 1QA, UK

²Australian Food Safety Centre, University of Tasmania, Hobart, Australia

³Fisheries Research Services Marine Laboratory, Aberdeen AB11 9DB, UK

⁴School of Aquaculture, University of Tasmania, Launceston, Australia

Phylogenetic and phenotypic analysis of cultivable marine bacteria isolated from laboratory cultures of two paralytic shellfish toxin-producing dinoflagellates, *Gymnodinium catenatum* and *Alexandrium tamarense*, showed the presence of a novel group of Gram-negative, aerobic, moderately halophilic and hydrocarbon-degrading bacteria, related to the genus *Marinobacter*. The strains, designated DG893^T, DG1136 and ATAM407-13, grew optimally in media with 3–6 % NaCl and at 25–30 °C, and all could utilize n-hexadecane and n-tetradecane as the sole carbon source. The strains had a 16S rRNA gene sequence similarity of 94.2–94.3 % to *Marinobacter hydrocarbonoclasticus* ATCC 27132, and a similarity of 97.5–97.8 % to the closest phylogenetically related type strain, *Marinobacter flavimaris* DSM 16070^T. DNA–DNA hybridization levels to *M. flavimaris* and other *Marinobacter* type strains were ≤42 %, while DNA–DNA reassociation values among DG893^T, DG1136 and ATAM407-13 were ≥83 %. The DNA G + C content was 54–55 mol% and the major isoprenoid quinone was ubiquinone-9. On the basis of phenotypic, chemotaxonomic, DNA–DNA hybridization and phylogenetic analysis, it is proposed that these three strains represent a novel species, *Marinobacter algicola* sp. nov. The type strain is DG893^T (=DSM 16394^T=NCIMB 14009^T).

The genus *Marinobacter* was first proposed in 1992 to accommodate a novel Gram-negative, aerobic, halophilic gammaproteobacterium capable of degrading a variety of hydrocarbons (Gauthier *et al.*, 1992). The type species, *Marinobacter hydrocarbonoclasticus*, was isolated from coastal waters, and since then a further 12 species of *Marinobacter* have been recognized. These were isolated from various locations such as an offshore oil-well head (Huu *et al.*, 1999), a coastal thermal spring (Shieh *et al.*, 2003), temperate (Yoon *et al.*, 2004) and Antarctic (Shivaji *et al.*,

2005) sea water, saline soils (Martín *et al.*, 2003) and marine sediments (Gorshkova *et al.*, 2003; Romanenko *et al.*, 2005). To date, only *Marinobacter bryozorum* 50-11^T has an identifiable biological source, having been isolated from homogenized bryozoan tissue (Romanenko *et al.*, 2005).

The bacterial flora associated with the dinoflagellates *Gymnodinium catenatum* Graham and *Alexandrium tamarense* (Lebour) Balech have been proposed to be involved in producing a potent suite of neurotoxins, the paralytic shellfish toxins (PSTs), produced by these algae (Gallacher *et al.*, 1997; Kodama *et al.*, 1990). As a part of the work to understand the relationship between the bacterial flora of PST-producing dinoflagellates and PST production, we identified bacterial isolates with a phylogenetic affiliation to *M. hydrocarbonoclasticus* in laboratory cultures of these dinoflagellates that formed a discrete phylogenetic cluster (Green *et al.*, 2004; Hold *et al.*, 2001). The phylogenetic clustering and frequency of occurrence in laboratory cultures was suggestive of a specific association between the dinoflagellate and the *M. hydrocarbonoclasticus*-like isolates (Green *et al.*, 2004; Hold *et al.*, 2001). Bacterial isolates

Published online ahead of print on 28 October 2005 as DOI 10.1099/ij.s.0.63447-0.

Abbreviation: PSTs, paralytic shellfish toxins.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains DG893^T, DG1136 and ATAM407-13 are AY258110, AY258116 and AJ294359, respectively.

A transmission electron micrograph of cells of DG893^T and a table detailing the cellular fatty acid composition of the three novel strains and the type strains of other *Marinobacter* species are available as supplementary material in IJSEM Online.

DG893^T and DG1136 were isolated from *G. catenatum* YC499B15 (recovered from the Yellow Sea, Korea) and *G. catenatum* GC21V (recovered from the Ria de Vigo, Spain), respectively, by serial dilution on a low-concentration marine agar (ZM/10; Green *et al.*, 2004). ATAM407-13 was isolated from *Alexandrium tamarense* NEPCC 407 (recovered from Jericho Beach, Vancouver, Canada) by culture on marine 2216 agar (Difco). The isolates were grown aerobically at 18–20 °C for 1–3 weeks and then stored frozen at –80 °C in marine broth (Difco) supplemented with 20 % glycerol (v/v).

Phenotypic examination of the isolates typically used colonies grown for 48–72 h on marine 2216 agar at 30 °C, streaked from ZM/10 marine agar slopes. *M. hydrocarbonoclasticus* NCIMB 1967 (=ATCC 27132), *Marinobacter aquaeolei* NCIMB 13596^T, *Marinobacter flavimaris* DSM 16070^T and *Marinobacter lipolyticus* NCIMB 13907^T were used for comparison, all maintained as above. Gram reaction and cell morphology of the strains were observed by light microscopy, and negatively stained whole cells were viewed by transmission electron microscopy on a JEOL-100SX (Beveridge *et al.*, 1994). Catalase and oxidase activity, utilization of Tweens 40 and 80 and casein and starch hydrolysis were also determined as described by Smibert & Krieg (1994). Nitrate reduction, indole production, arginine dihydrolase, urease, gelatin liquefaction, β -glucosidase and β -galactosidase activity were examined using API 20NE strips (Vitek-bioMérieux) inoculated with colonies suspended in 3 % NaCl (w/v). The ability of the isolates to utilize a range of organic substrates was determined using 96-well Biolog GN MicroPlates as described by Smith *et al.* (2002). The ability of the strains to utilize aliphatic hydrocarbons was determined by growing the strains on a synthetic sea-water agar (SM1; Yakimov *et al.*, 1998), varying only in that NH₄NO₃ was used as the nitrogen source. Bacteria were streaked onto the agar, and hydrocarbon was supplied in the vapour phase by adding 200 μ l of either n-tetradecane (Sigma) or n-hexadecane (Sigma) as the sole carbon source to a sterile piece of Whatman Qualitative 1 filter paper placed in the lid of the Petri dish. Growth was assessed after 3 weeks incubation at 25 °C. Utilization of hydrocarbon was assessed based on the amount of growth relative to cells streaked onto the same agar but without any added carbon source.

Strains DG893^T, DG1136 and ATAM407-13 were all similar in terms of their cell morphology, growth and phenotypic characteristics (see the species description, Table 1 and Supplementary Fig. S1 available in IJSEM Online). The three novel strains all demonstrated an ability to utilize a comparatively wide range of organic compounds as their sole carbon and energy source compared with those utilized by the other *Marinobacter* type strains examined. Discriminatory phenotypic characteristics are given in Table 1. DG893^T, DG1136 and ATAM407-13 were all positive for the utilization of Tweens 40 and 80, glycerol, dextrin, glycogen, glucose, methyl pyruvate, acetate, citrate, α -hydroxybutyrate,

β -hydroxybutyrate, DL-lactate, propionate, succinate, bromosuccinate, D-alanine, L-glutamate, L-leucine, L-proline and L-pyroglutamate. All strains were negative for the utilization of α -cyclodextrin, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, erythritol, L-fucose, D-galactose, gentiobiose, myo-inositol, α -lactose, α -D-lactose lactulose, D-mannose, D-melibiose, methyl β -D-glucoside, psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, p-hydroxyphenylacetic acid, itaconic acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, succinamic acid, glucuronamide, alaninamide, L-alanyl glycine, L-aspartic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-ornithine, DL-serine, L-threonine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenyl ethylamine, putrescine, α -DL-glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate.

Consistent with all recognized *Marinobacter* species, Na⁺ was essential for growth, and K⁺ would not suffice in its place. Of the NaCl concentrations tested [0, 1, 3, 6, 9, 12, 15, 20, 25 % (w/v)], growth was only observed at ≥ 1 % NaCl (w/v), and at up to 9 % for DG1136 and ATAM407-13, and up to 12 % for DG893^T. The range of pH over which all strains grew was consistent with other *Marinobacter* species. Growth was observed between pH 5 and 10 with an observed optimum of pH 7.5 for all strains. Anaerobic growth was observed to occur in all strains in the presence of nitrate and acetate, but not glucose. The temperatures examined for growth were 5, 10, 18, 25, 30, 37, 40 and 45 °C. The upper temperature at which growth was observed was 40 °C, while growth, albeit slow, occurred at 5 °C (Table 1). The optimal temperature range for growth of all strains was 25–30 °C.

Strains DG893^T, DG1136 and ATAM407-13 were capable of growth on n-tetradecane and n-hexadecane as their sole carbon source. Growth of ATAM407-13 on these two hydrocarbon sources was weaker than that of DG893^T and DG1136, and all three strains showed a preference for n-tetradecane. All strains showed an ability to grow on synthetic sea-water agar without any added carbon source, and formed shallow (<1 mm), colony-sized depressions in the agar; subsurface growth could be seen radiating out from beneath each colony. However, there was no evidence of softening of the agar surrounding the colony. This observation indicates the capacity for oligotrophic growth and/or the ability to utilize agar as a carbon source. Agarolytic activity was not otherwise observed in any of the strains when grown on more complex media, marine 2216 agar or the more dilute version, ZM/10 agar.

The 16S rRNA genes were sequenced as described by Green *et al.* (2004) and Hold *et al.* (2001). Taxonomic assignment based on the 16S rRNA gene sequences was initially performed using the Sequence Match facility of the Ribosomal Database Project II (Cole *et al.*, 2003). Multiple alignments were performed using CLUSTAL_X (Thompson *et al.*, 1997).

Table 1. Differential phenotypic characteristics between *Marinobacter algicola* sp. nov. and the type strains of selected *Marinobacter* species

Strains: 1, DG893^T, DG1136 and ATAM407-13; 2, *M. flavimaris* SW-145^T; 3, *M. lipolyticus* SM19^T; 4, *M. bryozoorum* 50-11^T; 5, *M. sediminum* R65^T; 6, *M. hydrocarbonoclasticus* ATCC 49840^T; 7, *M. aquaeolei* NCIMB 13596^T. Additional data for type strains were taken from Gauthier *et al.* (1992), Huu *et al.* (1999), Martín *et al.* (2003), Yoon *et al.* (2004) and Romanenko *et al.* (2005). +, Positive; –, negative; v, variable response.

Characteristic	1	2	3	4	5	6	7
Nitrate reduced to nitrite	–	+	–	+	–	+	+
Growth:							
Minimum temperature (°C)	5	4	15	7	4	10	13
Maximum temperature (°C)	40	45	40	42	42	45	50
NaCl range (%)	1–12*	1–20	1–15	1–18	0.5–18	1–20	1–20
Enzyme activity:							
Amylase	+	–	–	–	–	–	–
Urease	v†	–	–	–	–	–	+
Arginine dihydrolase	+	–	–	–	–	–	–
Utilization of:							
Glycerol	+	–	–	+	+	–	–
D-Fructose	+	+	+	–	–	–	–
D-Glucose	+	–	+	–	+	–	–
Maltose	+	–	+	–	–	–	–
D-Mannitol	v‡	–	+	–	–	–	–
Sucrose	–	–	–	+	+	+	+
Citric acid	+	–	–	–	–	+	–§
DL-Lactic acid	+	–	–	–	–	+	+
cis-Aconitic acid	+	–	–	–	–	+	+
D-Gluconic acid	+	+	+	–	–	–	–
L-Alanine	v†	–	–	–	–	–	+
L-Leucine	+	–	–	–	–	–	+
L-Phenylalanine	+	–	–	–	–	–	–
L-Proline	+	+	–	–	–	+	+
DNA G+C content (mol%)	54–55	58	57	59.6	56.5	53	56

*NaCl tolerance was 1–12% for strain DG893^T and 1–9% for strains DG1136 and ATAM407-13.

†DG893^T and DG1136 were both positive and ATAM407-13 was negative.

‡DG893^T was positive whereas DG1136 and ATAM407-13 were negative.

§Huu *et al.* (1999) reported citrate utilization as positive.

Alignments were manually corrected and ambiguous regions were masked from the analysis. The program PAUP4.0* (Swofford, 2001) was used to construct the distance tree based on the neighbour-joining algorithm (Saitou & Nei, 1987) according to the maximum-likelihood model, and bootstrap support for the inferred tree was established by resampling 1000 datasets by the neighbour-joining method (Saitou & Nei, 1987). *Alcanivorax borkumensis* SK2^T (GenBank accession no. Y12579) and *Alcanivorax venustensis* ISO4^T (GenBank accession no. AF328762) were used as the outgroup.

16S rRNA gene sequence analysis showed that DG893^T, DG1136 and ATAM407-13 were most closely related to *M. flavimaris* SW-145^T (97.8, 97.5 and 97.5%, respectively) and *M. lipolyticus* SM19^T (97.1, 97.2 and 97.3, respectively);

lower similarities were observed to the type strains of other *Marinobacter* species, such as *Marinobacter sediminum* R65^T (96.4–96.6%), *M. hydrocarbonoclasticus* ATCC 27132 (94.2–94.3%) and *M. aquaeolei* VT8^T (94.2–94.4%). 16S rRNA gene sequence similarity of DG893^T to DG1136 and ATAM407-13 was 99.3 and 99.7%, respectively, while DG1136 and ATAM407-13 had a sequence similarity of 99.9%. As the sequence divergence of DG893^T, DG1136 and ATAM407-13 from the closest phylogenetically related *Marinobacter* species (*M. flavimaris*) was > 2%, this supports the proposal that they may represent a novel species, and as DG893^T, DG1136 and ATAM407-13 were all ≥ 99.3% similar to one another, this is consistent with these strains belonging to a single species (Stackebrandt & Goebel, 1994). Phylogenetic analysis (Fig. 1) shows that DG893^T, DG1136 and ATAM407-13 form a distinct and

Fig. 1. Phylogenetic dendrogram based on the nearly complete 16S rRNA gene sequences of *Marinobacter algicola* sp. nov. and the type strains of other *Marinobacter* species (*Alcanivorax borkumensis* and *A. venustensis* were used as the outgroup; not shown). The tree was constructed by neighbour-joining using the maximum-likelihood model and bootstrap support (1000 replicates each) based on neighbour-joining. Bar, 0.01 nucleotide substitutions per site.

supported phylogenetic cluster separate from *M. flavimaris* SW-145^T and *M. lipolyticus* SM19^T, the two closest phylogenetic relatives. It also shows that the three strains comprise a coherent phylogenetic cluster within the genus *Marinobacter*.

Whole-genome DNA–DNA hybridization studies were conducted between DG893^T, DG1136, ATAM407-13, *M. hydrocarbonoclasticus* NCIMB 1967, *M. aquaeolei* NCIMB 13596^T, *M. lipolyticus* NCIMB 13907^T and *M. flavimaris* DSM 16070^T. Genomic DNA was extracted from washed, harvested cells grown in marine 2216 broth for 24 h at 30 °C according to the method described by Marmur (1961). Analysis of the G + C content and DNA–DNA hybridization were both performed essentially as described by Bowman *et al.* (1998). DG893^T showed DNA–DNA reassociation values of 35, 39, 42 and 35 % with *M. hydrocarbonoclasticus*, *M. aquaeolei*, *M. lipolyticus* and *M. flavimaris*, respectively. As these values were all < 70 %, this delineates DG893^T from the other *Marinobacter* type strains, and supports the proposal that DG893^T represents a novel species (Stackebrandt & Goebel, 1994). The DNA–DNA reassociation values of DG893^T with DG1136 and ATAM407-13 were 95 and 83 %, respectively, and the reassociation value of DG1136 to ATAM407-13 was 90 %. These DNA–DNA hybridization levels (> 70 %) support the proposal that DG893^T, DG1136 and ATAM407-13 represent a single species (Stackebrandt & Goebel, 1994). The DNA G + C content of DG893^T, DG1136 and ATAM407-13 was 55, 55 and 54 mol%, respectively,

consistent with values reported for the type strains of other *Marinobacter* species (Table 1).

Cellular fatty acid and isoprenoid quinone content were determined for cell mass grown on marine 2216 agar and broth, respectively, for 48 h at 30 °C. Fatty acid methyl esters were extracted and prepared according to standard protocols as described for the MIDI Microbial Identification System (Sasser, 1990). Cellular fatty acid content was analysed with the MIDI Microbial Identification system. Results are available as Supplementary Table S1 in IJSEM Online. The fatty acid composition was generally consistent with that of other *Marinobacter* type strains, although DG893^T, DG1136 and ATAM407-13 differed in having increased abundances of C_{16:1}ω7*c*/iso-C_{15:0} 2-OH (summed feature MIDI fatty acid methyl ester analysis), C_{16:0} 10-methyl and C_{18:1}ω7*c* and a reduced abundance of C_{16:1}ω9*c* (Supplementary Table S1). The isoprenoid quinone content of the three novel strains, with *M. hydrocarbonoclasticus* NCIMB 1967 and *M. flavimaris* DSM 16070^T as reference strains, was determined by reversed-phase HPLC using an Eclipse XDB-C18 column (Agilent Technologies), essentially as described by Komagata & Suzuki (1987). The principal isoprenoid quinone was shown to be ubiquinone-9 (Q-9), consistent with other *Marinobacter* type strains except *Marinobacter lutaoensis*, which contains ubiquinone-8 (Shieh *et al.*, 2003).

On the basis of 16S rRNA gene sequence analysis, DNA–DNA hybridization, phenotypic characteristics and chemotaxonomic properties, DG893^T, DG1136 and ATAM407-13 are proposed as three related strains of a novel species within the genus *Marinobacter*, for which the name *Marinobacter algicola* sp. nov. is proposed.

Description of *Marinobacter algicola* sp. nov.

Marinobacter algicola [al.gi'co.la. L. n. *alga* -ae an alga (seaweed, dinoflagellate etc.); L. suff. -cola from L. n. *incola* an inhabitant; N.L. fem. n. *algicola* an inhabitant of algae].

Gram-negative, oxidase- and catalase-positive, non-spore-forming rods, 1.6–2.5 × 0.45–0.55 μm, occurring as single cells, in pairs or as short chains of cells. Motile by means of a single, non-sheathed, polar flagellum. Colonies on marine 2216 agar at 25–30 °C after 72 h are cream, circular (1–2 mm), smooth, convex and shiny with entire edges. Older colonies change in shape from convex to umbonate and become light beige in the centre with pale margins. Growth requires Na⁺ and occurs across a range of total NaCl (w/v) of 1–9 % for DG1136 and ATAM407-13 and 1–12 % for DG893^T; optimal growth is between 3 and 6 % (w/v) NaCl. Growth occurs at 5–40 °C, with an optimal temperature of 25–30 °C, and over a pH range of 5–10, with an optimum of pH 7.5. Nitrate and nitrite are not reduced. Growth is aerobic, but anaerobic growth in the presence of nitrate and acetate, but not glucose, is observed. Tweens 40 and 80 and starch are hydrolysed, but gelatin and casein are not. Strains DG893^T and DG1136 are urease-positive, but ATAM407-13 is not. All strains identified to date are positive for arginine

dihydrolase activity, but negative for indole, β -glucosidase and β -galactosidase activity. All strains utilize n-hexadecane and n-tetradecane and other carbon sources as sole carbon and energy sources, as indicated in Table 1 and the main text. The predominant cellular fatty acids are C_{16:0} (22.0–25.5 %), C_{16:1 ω 7}/iso-C_{15:0} 2-OH (19.5–20.5 %), C_{18:1 ω 9}c (9.7–11.5 %) and C_{12:0} 3-OH (8.7–10.0 %). Q-9 is the principal isoprenoid quinone.

The type strain is DG893^T (=DSM 16394^T=NCIMB 14009^T). The DNA G+C content of the type strain is 55 mol%. Strains DG893^T and DG1136 were isolated from laboratory cultures of the dinoflagellate *Gymnodinium catenatum* (from Korea and Spain, respectively) and ATAM407-13 from the dinoflagellate *Alexandrium tamarense* (from Canada).

Acknowledgements

Strain ATAM407-13 was kindly donated for analysis by S. Gallacher and *G. catenatum* YC499B15 was kindly donated by Mr T. G. Park. We thank the National Collection of Plant-Pathogenic Bacteria (UK) for performing cellular fatty acid analysis. D. H. G. was supported in part by a post-doctoral fellowship from the New Zealand Foundation for Research, Science and Technology. This work was supported in part by funds to E. A. S. from Fisheries Research Services (UK).

References

- Beveridge, T. J., Popkin, T. J. & Cole, R. M. (1994). Electron microscopy. In *Methods for General and Molecular Bacteriology*, pp. 42–71. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Bowman, J. P., McCammon, S. A., Brown, J. L. & McMeekin, T. A. (1998). *Glaciecola punicea* gen. nov., sp. nov. and *Glaciecola pallidula* gen. nov., sp. nov.: psychrophilic bacteria from Antarctic sea-ice habitats. *Int J Syst Bacteriol* **48**, 1213–1222.
- Cole, J. R., Chai, B., Marsh, T. L. & 8 other authors (2003). The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* **31**, 442–443.
- Gallacher, S., Flynn, K. J., Franco, J. M., Brueggemann, E. E. & Hines, H. B. (1997). Evidence for production of paralytic shellfish toxins by bacteria associated with *Alexandrium* spp. (Dinophyta) in culture. *Appl Environ Microbiol* **63**, 239–245.
- Gauthier, M. J., Lafay, B., Christen, R., Fernandez, L., Acquaviva, M., Bonin, P. & Bertrand, J.-C. (1992). *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new extremely halotolerant, hydrocarbon-degrading marine bacterium. *Int J Syst Bacteriol* **42**, 568–576.
- Gorshkova, N. M., Ivanova, E. P., Sergeev, A. F., Zhukova, N. V., Alexeeva, Y., Wright, J. P., Nicolau, D. V., Mikhailov, V. V. & Christen, R. (2003). *Marinobacter excellens* sp. nov., isolated from sediments of the Sea of Japan. *Int J Syst Evol Microbiol* **53**, 2073–2078.
- Green, D. H., Llewellyn, L. E., Negri, A. P., Blackburn, S. I. & Bolch, C. J. S. (2004). Phylogenetic and functional diversity of the cultivable bacterial community associated with the paralytic shellfish poisoning dinoflagellate *Gymnodinium catenatum*. *FEMS Microbiol Ecol* **47**, 345–357.
- Hold, G. L., Smith, E. A., Rappe, M. S. & 7 other authors (2001). Characterisation of bacterial communities associated with toxic and non-toxic dinoflagellates: *Alexandrium* spp. and *Scrippsiella trochoidea*. *FEMS Microbiol Ecol* **37**, 161–173.
- Huu, N. B., Denner, E. B. M., Dang, T. C. H., Wanner, G. & Stan-Lotter, H. (1999). *Marinobacter aquaeolei* sp. nov., a halophilic bacterium isolated from a Vietnamese oil-producing well. *Int J Syst Bacteriol* **49**, 367–375.
- Kodama, M., Ogata, T., Sakamoto, S., Sato, S., Honda, T. & Miwatani, T. (1990). Production of paralytic shellfish toxins by a bacterium *Moraxella* sp. isolated from *Protogonyaulax tamarensis*. *Toxicon* **28**, 707–714.
- Komagata, K. & Suzuki, K. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–203.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J Mol Biol* **3**, 208–218.
- Martin, S., Márquez, M. C., Sánchez-Porro, C., Mellado, E., Arahál, D. R. & Ventosa, A. (2003). *Marinobacter lipolyticus* sp. nov., a novel moderate halophile with lipolytic activity. *Int J Syst Evol Microbiol* **53**, 1383–1387.
- Romanenko, L. A., Schumann, P., Rohde, M., Zhukova, N. V., Mikhailov, V. V. & Stackebrandt, E. (2005). *Marinobacter bryozoorum* sp. nov. and *Marinobacter sediminum* sp. nov., novel bacteria from the marine environment. *Int J Syst Evol Microbiol* **55**, 143–148.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*. Newark, DE: MIDI Inc.
- Shieh, W. Y., Jean, W. D., Lin, Y. T. & Tseng, M. (2003). *Marinobacter lutaoensis* sp. nov., a thermotolerant marine bacterium isolated from a coastal hot spring in Lutao, Taiwan. *Can J Microbiol* **49**, 244–252.
- Shivaji, S., Gupta, P., Chaturvedi, P., Suresh, K. & Delille, D. (2005). *Marinobacter maritimus* sp. nov., a psychrotolerant strain isolated from sea water off the subantarctic Kerguelen islands. *Int J Syst Evol Microbiol* **55**, 1453–1456.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Smith, E. A., Mackintosh, F. H., Grant, F. & Gallacher, S. (2002). Sodium channel blocking (SCB) activity and transformation of paralytic shellfish toxins (PST) by dinoflagellate-associated bacteria. *Aquat Microb Ecol* **29**, 1–9.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Swofford, D. L. (2001). *PAUP – Phylogenetic Analysis Using Parsimony and other Methods*, 4th edn. Sunderland, MA: Sinauer Associates.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Yakimov, M. M., Golyshin, P. N., Lang, S., Moore, E. R. B., Abraham, W.-R., Lünsdorf, H. & Timmis, K. N. (1998). *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *Int J Syst Bacteriol* **48**, 339–348.
- Yoon, J. H., Yeo, S. H., Kim, I. G. & Oh, T. K. (2004). *Marinobacter flavimaris* sp. nov. and *Marinobacter daepoensis* sp. nov., slightly halophilic organisms isolated from sea water of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **54**, 1799–1803.